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Manganese-iron Relationships Influencing Certain Blood Parameters and Organogenesis in Pregnant and Fetal Rabbits

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MANGANESE-IRON RELATIONSHIPS INFLUENCING CERTAIN BLOOD PARAMETERS AND ORGANOGENESIS IN PREGNANT AND FETAL RABBITS

BY

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IN PREGNANT AND FETAL RABBITS

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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INTRODUCTION

Studies conclusive of the role of manganese in normal metabolism have been possible only in recent years due to previous non-availability of precise quantitative techniques. Neutron activation analysis utilization has made possible measurement of as little as 3×10^{-5} ug/ml with 10 percent precision (Cotzias, 1958).

The importance of studying manganese involves its essentiality as a nutrient and its antagonism and interrelations with other essential dietary components.

Manganese was first shown to be essential for plant growth by McHargue (1923). Weinberg (1964) found that bacteria require manganese for growth, for culture longevity, and to synthesize antibiotics, bacteriophage, protective antigens, several enzymes and endospores. No other metal would satisfy this requirement. Underwood (1971) stated that manganese was essential to poultry to prevent perosis and nutritional chondrodystrophy. It was necessary in mammals for normal growth and reproductive function.

Specific biochemical roles for manganese have been found. Pyruvate carboxylase activity has been shown to depend upon manganese (Scrutton, Utler and Mildvan, 1966). Leach and Muenster (1962) demonstrated its essentiality in

mucopolysaccharide synthesis. Everson and Shrader (1968) disclosed that manganese had a role in glucose utilization.

Manganese has been found bound within erythrocytes and was thought to have a role in porphyrin metabolism (Borg and Cotzias, 1958). High dietary intake of manganese interferes with hemoglobin formation (Hartman, Matrone and Wise, 1955; Matrone, Hartman and Clawson, 1959; Cunningham, Wise and Barrick, 1966).

Effects of manganese deficiency on developing fetuses have been well documented (Everson, 1970; Underwood, 1971). However, effects of high maternal manganese levels altering fetal erythropoiesis have not been reported.

Underwood (1971) stressed the significance of the dietary manganese:iron ratio. Sato and Murata (1932) have shown that colostrum milk contains much more manganese than does normal milk, the first sample after parturition being especially high. These findings raise the question of manganese importance in fetal development, especially in erythropoiesis where iron is known to be of importance.

The objectives of this study are as follows:
a. to determine whether manganese is transported across the placenta against a concentration gradient as is iron (Larkin, Weintraub and Crosby, 1970); b. to ascertain if

high maternal blood manganese levels affect fetal erythropoiesis; c. to observe leukocyte formation alteration; d. to investigate whether fetal organogenesis is affected.

Certain parameters of both maternal and fetal functions were measured. Maternal red blood cells (RBC), white blood cells (WBC), packed cell volumes (PCV), hemoglobin levels, reticulocytes, white blood cell differentials, mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), RBC iron and manganese and plasma iron and manganese were compared. Fetal comparisons included RBC, WBC, PCV, hemoglobin, reticulocytes, MCH, MCV, MCHC, RBC iron and manganese, plasma iron and manganese and organ weights of liver, heart, lung and kidneys.

REVIEW OF LITERATURE

Metabolism

Manganese absorption has been studied extensively, especially in relation to absorption of other metals. Britton and Cotzias (1966) found that manganese was absorbed in proportion to the amount presented. They concluded that turnover depends extensively on dietary concentration. Pollack *et al.* (1965) determined absorption of manganese, cobalt and iron to be increased in iron deficient rats and attribute this to the similar chemistry of these elements. Their ionic radii are similar, and they coordinate with six ligands and form octahedral complexes.

However, Sahagian, Harding-Berlow and Perry (1967), using an *in vitro* perfusion method, described transmural movement of cobalt and manganese to be very rapid with little tissue retention and iron and zinc movement to be very slow with relatively high tissue retention.

Manganese fed to rats at levels of 500 or 1000 parts per million (ppm) was found to depress iron absorption (Hansard *et al.*, 1960). These authors inferred that manganese interferes with iron absorption to a greater extent than with iron utilization. Working with iron deficient animals, Forth (1970) found increased cobalt, manganese and zinc absorption along with iron and noted

an apparent competition.

Further, Lassiter, Morton and Miller (1970) have shown that more parenterally administered radiomanganese was excreted when rats were on a high calcium diet. High dietary phosphorus appeared to have no effect on parenterally administered manganese retention. Schaible and Bandemer (1942) and Wilgus and Patton (1939) had earlier shown this effect in birds, but their conclusion was based on adsorption of manganese by phosphates and carbonates in vitro, an effect thought to reduce soluble manganese.

Cotzias and Papavasiliou (1964) demonstrated several routes available for manganese excretion, all linked to the gastrointestinal canal. They further demonstrated that while the liver controls manganese flux following ordinary metabolic demand, the pancreas does so under extraordinary loads. Britton and Cotzias (1966) fed excess manganese and found the metal being absorbed in proportion to the amount presented. Their conclusion was that metabolism of this metal is regulated by its excretion rather than by both regulated absorption and excretion.

Papavasiliou, Miller and Cotzias (1966) contrasted intestinal excretion of manganese with renal excretion and concluded that the kidney excretes negligible amounts even during jaundice and after manganese loading. They

traced the route after absorption to the liver where manganese becomes localized in mitochondria followed by biliary discharge.

Rapid excretion of intravenously injected radiomanganese was found in bile, reaching a peak a few minutes after injection (Bertinchamps and Cotzias, 1968). Subsequent electrophoretic studies showed patterns with several peaks suggesting several chelates to be present.

An investigation determining the excretion sites in the gastrointestinal tract showed much higher amounts excreted by the duodenum and jejunum than by the terminal ileum (Bertinchamps, Miller and Cotzias, 1966). Cephalad segments responded more quickly to manganese loading suggesting these to be homeostatic end organs auxiliary to the liver.

Mahoney and Small (1968), investigating the biological half life of radiomanganese, found manganese disappearance from the body to be described by a curve having two exponential components. An average of 70 percent of injected material was eliminated by a "slow" pathway with an average value of 39 days. The half time for the "fast" component was four days. A low manganese intake increased the slow pathway percentage to 90 days.

It was shown by Hughes, Miller and Cotzias (1966) that cortisol affects manganese metabolism, but the

precise adrenal role remains uncertain. However, these authors feel syndromes in man similar to experimental manganese deficiency induceable by these hormones should be investigated.

Subcellular distribution of iron and manganese has been explored and has been found to be complementary. Nuclei and mitochondria were found to contain the highest manganese concentrations and lowest iron concentrations (Thiers and Vallee, 1957).

Ferrante (1961) stated that enzyme activation in cellular elements may be the prime function of manganese based on information indicating activation of a variety of enzymes by manganese *in vitro*. However, Cotzias and Papavasiliou (1962) indicated the existence of more than one chemical species of manganese in the body and argued that it was highly problematical whether *in vitro* manganese additives could duplicate exclusively such highly divergent natural chemical forms.

Scrutton, Utler and Mildvan (1966) demonstrated manganese in purified pyruvate carboxylase and quantitative analysis revealed 2.5 to 4.3 moles of manganese per mole of enzyme. Utilizing radiomanganese, good correlation was obtained between radioactivity and enzymatic activity. Manganese release occurred only after irreversible enzyme denaturation.

Underwood (1971) presented evidence for reduction of liver arginase in manganese deficient rats and rabbits. However, even though arginase activity was shown to increase *in vitro*, he was doubtful of *in vivo* activation by manganese.

Hypoglycemia has been shown to be an effect induced by manganese in an unusual diabetic (Rubenstein, Levin and Elliott, 1962). Manganese action on the pancreas was the favored explanation, for the effect was abolished following pancreatectomy. Manganese deficient guinea pigs demonstrated aplasia of all pancreatic cellular components with a resultant diabetic glucose tolerance curve. These effects were reversed by manganese administration (Everson and Schrader, 1968; Everson, 1970).

Manganese was first shown to be necessary for cartilage mucopolysaccharide synthesis by Leach and Muenster (1962). Grebner, Hall and Neufeld (1968) later demonstrated manganese catalysis of glucosamine-serine linkages. Everson (1970) demonstrated significant differences of acid mucopolysaccharides (AMPS) in cartilage of control and deficient guinea pigs. Depressed AMPS content of chicken egg shells has been shown with low manganese intake (Longstaff and Hill, 1970). Reduced epiphyseal cartilage mucopolysaccharide and hexosamine in lambs on low manganese diet has been demonstrated (Lassiter *et al.*, 1970).

Manganese has been implicated in lipid metabolism. Documentation for manganese-choline interaction has been presented, although the author states this to be little understood (Underwood, 1971). Curran (1954) presented evidence for manganese stimulation of hepatic cholesterol and fatty acid synthesis in rats, while Amdur, Rilling and Bloch (1957) found that manganese was necessary for conversion of mevalonic acid to squalene by mevalonic kinase.

Studying iron deficient subjects, Mahoney and Small (1968) suggested an interrelation between manganese and iron metabolism. Experimental data presented by Titus, Cave and Hughes (1928) indicated that manganese added to milk-iron diets produced quicker responses in hemoglobin building than non-supplemented diets. Beard, Baker and Myers (1931) demonstrated that iron supplemented with various trace metals, including manganese, enhances maturation of erythrocytes. Myers and Beard (1931) stated that iron was essential in hemoglobin regeneration in nutritional anemia, while copper, nickel, germanium, manganese, arsenic, titanium, zinc, rubidium, chromium, vanadium, selenium and mercury action was probably catalytic.

Working with lambs receiving high manganese supplements, Hartman, Matrone and Wise (1955) discovered that hemoglobin levels were lower than controls. This was

thought to be due to either interference with iron absorption, with hemoglobin formation or a combination. Cotzias (1958) suggested an indirect role of manganese in hematopoiesis.

Borg and Cotzias (1958) found a firmly bound manganese component in human and rabbit erythrocytes and suggested that manganese had a role in porphyrin metabolism as selective concentrations of ^{54}Mn were found in red cell fractions of blood. They showed that the metal was non-dialysable, non-exchangeable and not available for chelation with disodium ethylenediamine tetraacetic acid (EDTA).

Investigating manganese-iron antagonism, Matrone, Hartman and Clawson (1959) found high manganese supplements depressed hemoglobin formation, but high iron supplements reversed this effect. However, Cunningham, Wise and Barrick (1962) did not find any effect on hemoglobin formation in calves with high manganese supplements. Cunningham, Wise and Barrick (1966), in conflict to their earlier findings, showed depressed hemoglobin levels in calves fed manganese in high levels. Equal amounts of feed were given to all calves in the second study while ad libitum feeding characterized the first.

Mackiewicz (1965a) stated that manganese administered to anemic rats had no influence on the course of the

anemia. However, in a subsequent study, she found iron supplemented with manganese was more effective in its erythropoietic effect than iron alone (Mackiewicz, 1965b).

Cotzias, Miller and Edwards (1966), searching for a manganese porphyrin, suggested that their experiments indicated the incorporation of stable manganese into the heme moiety of erythrocytes. Animals made anemic by bleeding or by iron deficient diet increased the amounts of iron, manganese and cobalt absorbed (Pollack et al., 1965).

McNary (1960) reported the presence of manganese in neutrophils and eosinophils, but the concentration in other leukocytes or platelets has not been described.

Arthritics have been found to have high erythrocyte concentrations of manganese with slow turnover rates. A correlation with abnormalities of mucoprotein and mucopolysaccharide metabolism exhibited by arthritics was suggested by these authors (Cotzias et al., 1968).

Meiri and Rahamimoff (1972) have shown that manganese ions inhibit neuromuscular transmission. Alterations in manganese concentrations have been associated with certain neurological disorders (Mena et al., 1967, 1969; Cotzias et al., 1968). The possibility does exist that certain synapses may possess a high sensitivity for manganese ions.

Newland and Davis (1961), feeding high levels of manganese to gravid gilts, found no significant difference in fetal growth, although fetuses from high manganese level sows did contain higher manganese concentrations. Hansard (1970) found that manganese accumulated in fetuses during the final trimester. Gamble et al. (1971), 168 hours after injecting ^{54}Mn, found 25.7 percent of the retained metal in the conception products, 87 percent of which was in the developing fetuses.

Placental manganese transport mechanisms have yet to be elucidated, but iron was found to be transported to placenta cells by transferrin-iron complex. Placental cells bind the complex, utilize the iron, and release the transferrin. These cells were able to take up iron against concentration gradients and transport it in a unidirectional vectorial process (Larkin, Weintraub and Crosby, 1970).

Levels and Storage

Tipton, Stewart and Martin (1966), in a 30 day study of two normal human subjects, found average daily intakes of manganese were 4.39 mg and 3.45 mg. The amounts found in feces were 1.21 mg and 2.59 mg, leaving a balance of 3.45 and 0.54 mg. Underwood (1971) reported that bones, liver, kidneys, pancreas and pituitary gland

carried higher manganese concentrations. Skeletal muscles were among the lowest. Cotzias (1958) stated that a 70 kg man contains 12 to 20 mg manganese. Schroeder, Balassa and Tipton (1966) found that body distribution of manganese was wide with particular organs having characteristic concentrations which varied little within or among species or with age. Underwood (1971) further noted that skeletal manganese does not constitute an important mobilizeable store.

Values reported in the literature for blood manganese content vary widely. Values obtained from early studies reviewed by Ferrante (1961) ranged from 2.4 to 13 ug/100 ml. Later work using more accurate neutron activation analysis reported by Cotzias, Miller and Edwards (1966) and Papavasiliou, Miller and Cotzias (1966) found 8.44 ug/liter and 9.84 ug/liter respectively. This was partitioned to obtain values of 1.42 ug/liter for serum and 23.57 ug/liter for erythrocytes.

Sawhney and Kehar (1961) investigated manganese content of blood from 12 bullocks and found 0.0180 to 0.0184 mg/100 ml. This was in contrast to neutron activation analysis of manganese in rabbit blood by Papavasiliou and Cotzias (1961) who found only 6.85 ug/liter.

Conflicting data exists concerning liver storage of manganese. Underwood (1971) reported work of several

investigators showing no reserve stores of manganese in liver tissue, but Howes and Dyer (1971) demonstrated that newborn calves preferentially utilized hepatic tissue to store manganese.

Hughes, Miller and Cotzias (1966) showed that manganese was shifted from liver to carcass tissues following administration of glucocorticoids or adrenocorticotropic hormone. They further demonstrated that manganese concentrations were not altered following adrenalectomy except in animals receiving diets supplemented with high level manganese.

Anke and Groppel (1970) fed ^{52}Mn to hens and cows, finding that most of this isotope was transported to bones and ovaries for storage, but a transient accumulation was found in liver, feathers, skeletal muscle and kidneys.

Requirements

Manganese requirements depend upon species, criteria of adequacy, chemical form and other constituents in the diet (Underwood, 1971). Birds are known to have a higher requirement than mammals.

Wachtel, Elvehjem and Hart (1943) found that 0.1 to 0.2 ppm manganese added to a milk diet was inadequate for growth in mice, rats and rabbits. High phosphorus in relation to calcium increases the requirement.

Deficiency

Everson (1970) found that deficiency affects growth, viability of offspring, skeletal development, coordination, fertility and arginase activity. Underwood (1971) stated that these manifestations were similar in all species, although expression varies. His review of the literature failed to produce unequivocal evidence of uncomplicated deficiency in grazing livestock, although deficiency does occur naturally in certain diets fed to pigs and poultry.

Lassiter et al. (1970) found that early weaned lambs receiving a purified diet with less than one ppm manganese for five months exhibited bone changes demonstrated by tibias which were lighter, shorter, had reduced volumes and lower breaking strength. Longstaff and Hill (1970), feeding low-manganese-high-phosphorus diets to chickens observed no changes in shell matrix as a whole but a markedly depressed AMPS content of matrix.

Maternal diets deficient in manganese resulted in increased incidence of stillbirths with many young failing to survive more than three or four days in guinea pigs (Everson, 1970). In a study utilizing goats receiving low manganese diets, 23 percent of the treatment animals aborted, while no abortions occurred in control groups (Anke and Groppel, 1970).

Offspring of manganese deficient guinea pigs studied by Everson (1970) had failure of normal otolith development which was not correctable by manganese supplements. This developmental failure resulted in ataxia in both poultry and mammals (Underwood, 1971).

Anke and Groppel (1970) found that deficient female goats had later estrus, weak symptoms of estrus, and required more inseminations to become pregnant. Underwood (1971) cited early work demonstrating defective ovulation, testicular degeneration and increased infant mortality in deficient animals. However, there was no indication of the precise locus. Manganese was not normally present in high concentration in gonadal tissue.

Toxicity

Cotzias (1958) reported natural manganese poisoning occurred among miners working with manganese ores. Characteristics of chronic poisoning were locura manganica which resembled schizophrenia and permanent neurologic (extrapyramidal) disorder similar to Parkinson's disease. Mode of entry was via the lungs and gastrointestinal tract.

Gallup and Norris (1939) reported rat growth was unaffected by 1000 to 2000 ppm dietary manganese and hens tolerated 1000 ppm. Grummer et al. (1950) stated that 500 ppm manganese in diets of growing pigs retarded growth and

depressed appetite. Cunningham et al. (1966) found no effect in calves with 820 ppm, but depressed feed intake and weight gain with 2460 and 4920 ppm. Increased manganese caused a small but significant depression of hemoglobin formation. Therefore, manganese rates in the lower group of toxic trace elements.

Underwood (1971) indicated that the manganese:iron dietary ratio had a wider significance than was commonly thought. Matrone, Hartman and Clawson (1959) found that 1250 and 2000 ppm manganese caused depressed hemoglobin synthesis in rabbits and baby pigs, but was reversed by 400 ppm iron supplements. They assumed an interference with iron absorption rather than with hematopoiesis.

Mena et al. (1970) suggested that chronic manganese poisoning could injure the dopaminergic components of the extrapyramidal system in a manner paralleling Parkinson's syndrome. Patients receiving L-Dopa (L-Dihydroxyphenylalanine) showed striking reduction or disappearance of rigidity and hypokinesia, marked improvement of postural reflexes and restitution of balance.

EXPERIMENTAL PROCEDURE

Experimental Animals

Twenty-one Dutch-belted female rabbits weighing 1950 to 2650 grams were utilized in these investigations. All were active, alert and apparently disease free. Each animal was penned individually in stainless steel batteries and provided with water and pelletized rabbit ration[1] *ad libitum*. Animals were bred to Dutch-belted males. Each animal was assigned to either a saline control group (Group I) or to one of two treatment groups (Groups II and III).

Stock treatment solution was prepared from research grade $MnCl_2$-4 H_2O and double-distilled deionized water to contain 2.0 mg Mn^{+2}/ml with correction made for water of hydration. Group I (control) does received 10 ml 0.85% NaCl, Group II received 2.5 mg/Kg body weight in 10 ml solution and Group III received 10 mg/Kg body weight in 10 ml solution. The injections were administered intraperitoneally immediately following withdrawal of pretreatment blood samples.

In order to prevent contamination with metals to be measured, all glassware was thoroughly washed, rinsed twice in double-distilled deionized water, soaked in 3N

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[1]Ralston Purina Co., St. Louis, Missouri, 63188.

HCl for four hours, rinsed in double-distilled deionized water and oven-dryed. Syringes and needles were treated as glassware except for only a brief rinse in 3N HCl.

All doe blood collections were accomplished by cardiac puncture employing heparinized glass syringes and 18-gauge needles. Samples were immediately placed in glass tubes containing 500 units sodium heparin and stoppered. Approximately 12 ml of blood was withdrawn. Pretreatment samples were collected on Day 22 to Day 24 of pregnancy. Final samples were obtained on either Day 29 or Day 30 of pregnancy prior to sacrificing.

Animals were sacrificed on Day 29 or Day 30 of pregnancy by massive cardiac air embolism. The abdomen was immediately opened and fetuses removed, tagged and placed in a stainless steel container suspended in a second container containing warm water to prevent death by exposure before blood samples could be collected.

Blood samples were obtained from fetuses by severing the carotid artery and collecting the blood in glass tubes containing 500 units sodium heparin and then stoppered. It was found that even though the blood sample was small (approximately 2 ml) large amounts of anticoagulant were necessary to prevent coagulation. The fetuses were then disected as outlined by Thibodeau (1967). The following organs were removed: left lung; heart; right

kidney; and liver. These organs were weighed on a Sartorius balance sensitive to one ten-thousandth gram.

Hemogram Determinations

Red and white blood cells were enumerated in duplicate utilizing the Coulter Counter Model F[2]. Dilutions were prepared with a Coulter Counter Dual-Diluter[3] using Isoton[4] as the diluent and Zap-Isoton[5] as a lysing agent. Red cell counts were corrected employing the Coulter Counter Coincidence Correction Chart.[6]

Duplicate packed cell volumes were determined employing the microhematocrit technique.[7] Duplicate hemoglobin determinations were obtained by the cyanmethemoglobin method[8] using Hycel[R] hemoglobin standard[9], and a Beckman Grating Spectrophotometer Model DB-G.[10]

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[2]Coulter Electronics, Inc., Hialeah, Florida.

[3]Coulter Electronics, Inc., Hialeah, Florida.

[4]Coulter Electronics, Inc., Hialeah, Florida.

[5]Coulter Diagnostics, Inc., Miami Springs, Florida.

[6]Coulter Electronics, Inc., Hialeah, Florida.

[7]Clay Adams, Inc., New York, New York, 10010.

[8]Cyanmethemoglobin Determinations, Hycel, Inc., Houston, Texas, 77036.

[9]Hycel, Inc., Houston, Texas, 77036.

[10]Beckman Instruments, Inc., Fullerton, California, 92634.

The Wintrobe indices, mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were calculated using the following formulae:

$$\text{MCH (micromicrograms)} = \frac{\text{g hemoglobin/100 ml blood x 10}}{\text{erythrocytes, million/cubic mm}}$$

$$\text{MCV (cubic microns)} = \frac{\text{packed cell volume x 10}}{\text{erythrocytes, million/cubic mm}}$$

$$\text{MCHC (percent)} = \frac{\text{g hemoglobin/100 ml blood x 100}}{\text{packed cell volume, percent}}$$

All hemogram parameters of both doe and fetuses were completed within four hours after obtaining the blood samples. At this time blood smear slides were prepared and stained with methylene blue for reticulocyte determinations according to the procedure of Brecher (1949). One thousand erythrocytes were counted utilizing an ocular grid and oil immersion lens. Blood smear slides were prepared for leukocyte differential determinations using Wright's stain in the procedure outlined by Davidsohn and Nelson (1969). Two hundred leukocytic cells were counted employing an oil immersion lens.

Iron and Manganese Determinations

Fetal blood was pooled by litter for iron and manganese determinations. Pre-treatment and final doe blood samples were analyzed individually. Red blood cells and plasma were separated and prepared for analysis according

to the method recommended by Wade (1972). Sample iron and manganese was determined employing a Perkin-Elmer Atomic Absorption Spectrophotometer, Model 303.[11] Standards utilized for iron were 1, 5, 10, 15, 20 and 25 ppm. Manganese standard concentrations were 0.1, 0.2 and 0.5 ppm. Interpolated values for calculating sample concentrations were obtained using an Olivetti Underwood Programma 101[12] with the following formula:

$$y(x) = \frac{1}{x_0 - x_1} (y_0 - y_1)\ x - y_0 x_1 + y_1 x_0$$

Where x_0 and x_1 equal standard concentrations, y_0 and y_1 equal standard absorbance values and x and y equal sample concentration and absorbance, respectively.

Data Analysis

Analysis of data was completed using an Olivetti Underwood Programma 101. The analysis of variance was used as the test of statistical significance for all data. Red blood cells, white blood cells, packed cell volumes, hemoglobin levels, reticulocytes, white blood cell differentials, MCH, MCV, MCHC, red blood cell iron and manganese and plasma iron and manganese were compared for does. Fetal comparisons were made for red blood cells, white

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[11] Perkin-Elmer Corp., Norwalk, Connecticut.

[12] Olivetti Underwood, One Park Avenue, New York, New York, 10016.

blood cells, packed cell volumes, hemoglobin, reticulocytes, MCH, MCV, MCHC, red blood cell iron and manganese and plasma iron and manganese. Also, wet weights of fetal liver, heart, left lung and right kidney were compared as the percentage of total body weight.

Results of statistical computations are shown in tables 1 and 2.

TABLE 1. ANALYSIS OF VARIANCE FOR DOE DATA

Basis	Source	d.f.	Sum of Squares	Mean Square	F Ratio
Red Blood Cells	Treatment	5	6.077	1.215	0.833^{NS}
	Error	36	52.507	1.458	
	Total	41	58.584		
White Blood Cells	Treatment	5	1856.444	371.288	0.899^{NS}
	Error	12	4952.668	412.722	
	Total	17	6809.112		
Packed Cell Volume	Treatment	2	2.595	1.298	0.160^{NS}
	Error	18	145.714	8.095	
	Total	20	148.310		
Hemoglobin	Treatment	2	0.292	0.146	0.144^{NS}
	Error	18	18.165	1.009	
	Total	20	18.458		
Reticulocytes	Treatment	5	3.630	0.726	0.814^{NS}
	Error	36	32.088	0.891	
	Total	41	35.718		
Mean Corpuscular Hemoglobin	Treatment	5	42.687	8.537	1.074^{NS}
	Error	36	285.974	7.943	
	Total	41	328.661		

TABLE 1 CONTINUED

Basis	Source	d.f.	Sum of Squares	Mean Square	F Ratio
Mean Corpuscular Volume	Treatment	5	142.408	28.481	0.343^{NS}
	Error	36	2985.211	82.922	
	Total	41	3127.619		
Mean Corpuscular Hemoglobin Concentration	Treatment	5	15.208	3.041	0.882^{NS}
	Error	36	123.999	3.444	
	Total	41	139.207		
Red Blood Cell Manganese	Treatment	5	26.584	5.316	1.049^{NS}
	Error	28	141.911	5.068	
	Total	33	168.495		
Plasma Manganese	Treatment	2	1.764	0.882	0.142^{NS}
	Error	17	105.444	6.203	
	Total	19	107.208		
Red Blood Cell Iron	Treatment	2	81.117	40.559	2.399^{NS}
	Error	17	287.410	16.906	
	Total	19	368.527		
Plasma Iron	Treatment	5	0.331	0.066	1.421^{NS}
	Error	33	1.539	0.046	
	Total	38	1.870		

^{NS}Not Significant.

TABLE 2. ANALYSIS OF VARIANCE FOR FETUS DATA

Basis	Source	d.f.	Sum of Squares	Mean Square	F Ratio
Red Blood Cells	Treatment	2	0.317	0.158	0.374^{NS}
	Error	129	54.473	0.422	
	Total	131	54.790		
White Blood Cells	Treatment	2	198.130	99.065	26.214**
	Error	129	487.527	3.779	
	Total	131	685.657		
Packed Cell Volume	Treatment	2	476.439	238.219	8.651**
	Error	129	3552.082	27.535	
	Total	131	4028.521		
Hemoglobin	Treatment	2	19.759	9.879	5.161**
	Error	129	246.967	1.914	
	Total	131	266.726		
Reticulocytes	Treatment	2	316.641	158.320	14.731**
	Error	128	1375.341	10.747	
	Total	130	1692.282		
Mean Corpuscular Hemoglobin	Treatment	2	159.892	79.946	9.833**
	Error	129	1048.828	8.130	
	Total	131	1208.720		

TABLE 2 CONTINUED

Basis	Source	d.f.	Sum of Squares	Mean Square	F Ratio
Mean Corpuscular Volume	Treatment	2	3038.104	1519.052	14.571**
	Error	129	13448.049	104.248	
	Total	131	16486.153		
Mean Corpuscular Hemoglobin Concentration	Treatment	2	15.156	7.578	5.447**
	Error	129	179.536	1.391	
	Total	131	194.692		
Red Blood Cell Manganese	Treatment	2	704.389	352.194	19.358**
	Error	18	327.492	18.194	
	Total	20	1031.881		
Plasma Manganese	Treatment	2	18.648	9.324	1.176^{NS}
	Error	17	134.742	7.926	
	Total	19	153.390		
Red Blood Cell Iron	Treatment	2	110.766	55.383	1.995^{NS}
	Error	18	499.548	27.753	
	Total	20	610.314		
Plasma Iron	Treatment	2	0.165	0.083	0.967^{NS}
	Error	18	1.541	0.085	
	Total	20	1.706		

TABLE 2 CONTINUED

Basis	Source	d.f.	Sum of Squares	Mean Square	F Ratio
Total Body Weight	Treatment	2	694.314	347.157	6.812**
	Error	135	6879.542	50.959	
	Total	137	7573.856		
Heart	Treatment	2	0.259	0.129	20.855**
	Error	135	0.847	0.006	
	Total	137	1.106		
Liver	Treatment	2	15.323	7.661	14.187**
	Error	131	70.769	0.540	
	Total	133	86.092		
Kidney	Treatment	2	0.114	0.057	17.273**
	Error	135	0.451	0.003	
	Total	137	0.565		
Lung	Treatment	2	1.147	0.573	21.882**
	Error	135	3.542	0.026	
	Total	137	4.689		

**P < .01.

NS Not Significant.

RESULTS AND DISCUSSION

Analysis of variance was employed to evaluate treatment effects. Results of these analyses are presented in tables 1 and 2.

Significant differences could not be shown between control and treatment does. However, both control and treatment groups demonstrated decreased packed cell volumes, hemoglobin and red blood cell iron during the final week of pregnancy. Elevated blood volume and iron storage depletion are normal physiological alterations during pregnancy which account for these changes (Guyton, 1971).

Red blood cell enumerations of fetuses in treatment groups did not differ significantly from controls. Reticulocyte counts were elevated (highly significantly) in treatment groups compared to controls. Increased production and release of new erythrocytes by erythropoietic tissues is indicated (Davidsohn and Nelson, 1969). Tissue hypoxia and depressed oxygen transport does elicit such a response.

Fetal packed cell volumes were significantly decreased in treatment groups although not significantly between high and low manganese treatments. Hemoglobin values were also depressed in treatment groups (highly

significantly). Lowered packed cell volumes and hemoglobin levels indicate erythrocytic dilution by tissue fluid influx. This attests to the fact that treated animals had a decreased ability to transport oxygen.

Mean corpuscular volumes and mean corpuscular hemoglobin levels showed highly significant decreases in treatment animals which is consistent with microcytic, hypochromic anemias. Mean corpuscular hemoglobin concentrations were increased (highly significantly) in treated animals. These are indicative of blood volume reduction which occurs in spherocytosis, an occasional occurrence in newborn mammals (Platt, 1969).

Red blood cell enumerations and plasma iron levels were not significantly different in treatment groups. Plasma manganese concentrations were also unchanged. However, red blood cell manganese levels were elevated over two-fold in treated animals, a highly significant statistical difference. The interference of manganese in normal hemoglobin formation accounts for the overall decrease of hemoglobin values. This is in agreement with the findings of Hartman *et al.* (1955), Matrone *et al.* (1959) and Cunningham *et al.* (1966).

Highly significant decreases were found in circulating leukocyte numbers in treatment groups. Leukopenia was a common laboratory finding in chronic

manganese poisoning (Cotzias, 1958). The complete leukocytic alterations of this leukopenia could not be determined without white blood cell differential and bone marrow studies. Interestingly, disseminated lupus erythematosus, a disease characterized by leukopenia, was improved by manganese injection (Comens, 1956).

The wet weights of fetal carcasses were significantly reduced in treated animals. Cotzias (1958) reported decreased body weight to be symptomatic of excessive manganese intake, presumably due to its general toxicity.

Fetal heart weights, computed as percent of total body weight, were highly significantly decreased in treated animals. The heart has been shown to contain very small amounts of manganese, both naturally and following injection (Schroeder, Balassa and Tipton, 1966). Von Oettingen (1935) reported histological studies showing cardiac degeneration. In the absence of definitive histological investigations, the weight changes were explained by manganese interference with proliferation of normal tissue.

Lung tissue has been found to contain very small amounts of manganese, both normally and following large manganese injections (Schroeder, Balassa and Tipton, 1966; Underwood, 1971). Fetal lung weights were

increased (highly significantly) in treated animals. Due to the small manganese concentrations found in lungs after injection, it is doubtful if this increase was due to proliferation of macrophages. More likely, it was due to a combination of fatty degeneration as found in other tissues (von Oettingen, 1935) and to fetal hypoxic alterations.

Fetal liver weights were increased significantly. Due to hepatic excretory functions in manganese turnover, these increased liver weights were probably a manifestation of elevated work load (Cotzias, 1958; Underwood, 1971). Histologically, liver of manganese treated animals has been shown to be in a state of fatty degeneration (von Oettingen, 1935).

Kidney weights were significantly depressed. Kidneys contain large concentrations of manganese, second only to the liver and pancreas (Schroeder, Balassa and Tipton, 1966). Kidneys of manganese treated animals were nephritic, cirrhotic and degenerating (von Oettingen, 1935). The obviously toxic effects of manganese on adult organs probably interfered in the developmental processes occuring in the fetus.

Therefore, there are manganese-iron relationships altering hemogram parameters, plasma manganese and iron concentrations and total body and certain organ

weights.

SUMMARY

Manganese, a trace element, has been known to be essential for animal nutrition since 1931. Its deficiency in pregnant animals causes profound fetal developmental changes, especially in ossification processes. Excess manganese interferes with iron metabolism. These investigations attempted clarification of high maternal manganese effects on placental iron transport, fetal erythropoiesis, fetal leukocyte formation and organogenesis.

Twenty-one Dutch-belted female rabbits and 138 fetuses were utilized in these studies. Animals were randomly assigned to a control group (Group I), a low level manganese treatment group (Group II) or a high level manganese treatment group (Group III). Group I received 10 ml 0.85% NaCl, Group II received 2.5 mg manganese per kilogram and Group III received 10 mg manganese per kilogram intraperitoneally on Day 22 to Day 24 of pregnancy. Blood samples were obtained immediately before treatment and sacrificing. Red blood cells (RBC) and white blood cells (WBC) were enumerated electronically. Also measured were packed cell volumes (PCV), hemoglobin levels, reticulocytes, white blood cell differentials, mean corpuscular hemoglobin (MCH), mean

corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), red blood cell and plasma iron and red blood cell and plasma manganese concentrations.

Fetal blood sample analysis included RBC, WBC, PCV, nemoglobin levels, reticulocytes, MCH, MCV, MCHC, red blood cell and plasma iron and red blood cell and plasma manganese levels. Wet weights of fetal liver, heart, left lung, right kidney and total body weights were measured.

Utilizing analysis of variance no significant differences were demonstrated in the doe data. However, fetal data showed significant alterations in treated animals. Reticulocyte numbers were increased highly significantly while red blood cells did not differ between groups. Hemoglobin levels were depressed indicating that even though red blood cell numbers were the same, oxygen carrying capability was lowered. Hemoglobin weight per cell was decreased as evidenced by lowered MCH values although MCHC concentrations indicated hemoglobin percent elevation, probably due to spherocytosis. PCV values were decreased indicating tissue fluid movement into the cardiovascular system as RBC values remained the same. White blood cell formation was depressed highly significantly.

No difference was demonstrated in red blood cell

iron, plasma iron or plasma manganese levels. However, red blood cell manganese concentrations were doubled, a highly significant difference.

Fetal body weight was highly significantly reduced. Also fetal heart and kidney had lowered organ weights while liver and lung increased highly significantly.

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APPENDIX

RAW DATA TABLE I

PRE-TREATMENT SAMPLES

Animal	Weight g	RBC[1]	WBC[2]	PCV[3] %	Hb[4] g%	Reticulocytes[5]
Group I (Control)						
1	2050	9.17	4.84	32.0	10.9	1.7
2	2050	5.42	5.49	35.5	11.5	2.3
3	2270	6.20	6.16	38.0	11.7	2.3
4	2260	5.89	8.55	38.0	11.4	2.1
5	1965	6.42	6.76	42.5	11.0	1.6
6	2290	4.66	5.53	33.5	10.3	4.0
7	2100	5.39	3.00	27.0	9.9	1.4
Mean	2141	6.16	5.76	35.2	11.0	2.2
Group II						
1	2130	6.44	5.40	38.0	12.4	1.5
2	2210	5.45	4.42	37.0	12.3	3.0
3	2320	5.15	4.43	33.0	11.5	2.7
4	2260	5.71	5.41	36.5	12.2	3.0
5	2350	5.73	3.64	37.0	12.2	1.6
6	2330	6.11	5.58	37.5	12.8	2.7
7	1880	5.65	7.40	35.5	11.5	1.6
Mean	2211	5.75	5.18	36.4	12.1	2.3
Group III						
1	2410	5.55	5.31	36.0	11.7	2.5
2	2600	5.32	5.61	34.5	11.2	2.4
3	2490	6.16	6.40	39.5	12.9	1.8
4	2660	5.08	3.76	32.5	10.6	4.1
5	2300	5.85	5.57	36.0	12.4	2.9
6	2540	5.80	5.79	38.5	13.3	1.6
7	2150	5.88	6.22	37.0	12.0	2.6
Mean	2450	5.66	5.52	36.3	12.0	2.6

[1] Red Blood Cells x $10^6/mm^3$.

[2] White Blood Cells x $10^4/mm^3$.

[3] Packed Cell Volume.

[4] Hemoglobin.

[5] Reticulocytes per 1000 erythrocytes.

RAW DATA TABLE II

PRE-TREATMENT SAMPLES

Animal	RBC Fe mg%	Plasma Fe mg%	RBC Mn ug%	Plasma Mn ug%	MCH[1] uug	MCV[2] u^3	MCHC[3] %
Group I (Control)							
1	32.81	0.48	8.11	4.40	11.89	34.89	34.06
2	32.65	0.31	6.06	7.32	21.24	65.56	32.39
3	37.52	0.19	4.40	4.17	18.87	61.29	30.79
4	35.96	0.18	5.51	3.93	19.34	64.46	30.00
5	36.73	0.31	6.14	5.03	17.13	66.20	25.88
6	28.33	0.61	4.09	6.14	22.08	71.81	30.75
7	26.64	0.23	3.80	5.45	18.37	50.09	36.67
Mean	32.94	0.33	5.44	5.20	18.41	59.18	31.50
Group II							
1	36.90	0.64	6.45	7.48	19.27	59.05	32.63
2	32.03	0.80	0.00	7.24	22.57	67.89	33.24
3	28.74	0.19	0.00	6.03	22.33	64.08	34.85
4	29.83	0.15	3.38	7.19	21.37	63.92	33.42
5	55.61	0.36	5.99	11.10	21.27	64.52	32.97
6	34.58	0.78	3.22	6.03	20.93	61.32	34.13
7	29.66	0.40	2.89	5.45	20.35	62.83	32.39
Mean	35.33	0.47	3.13	7.21	21.15	63.37	33.37
Group III							
1	34.72	0.09	7.75	4.00	21.06	64.81	32.50
2	25.12	0.23	10.35	0.00	21.03	64.79	32.46
3	38.18	0.09	8.92	4.04	20.94	64.12	32.66
4	--	--	--	--	20.84	63.91	32.61
5	31.56	0.15	0.00	5.77	21.18	61.48	34.44
6	--	--	--	--	22.91	66.32	34.54
7	31.77	0.22	4.04	6.03	20.39	62.87	32.43
Mean	32.27	0.15	6.21	3.96	21.19	64.04	33.09

[1]Mean Corpuscular Hemoglobin.

[2]Mean Corpuscular Volume.

[3]Mean Corpuscular Hemoglobin Concentration.

RAW DATA TABLE III

FINAL SAMPLES

Animal	Weight g	RBC[1]	WBC[2]	PCV[3] %	Hb[4] g%	Reticulocytes[5]
Group I (Control)						
1	2050	9.11	3.91	31.0	9.8	1.1
2	2041	4.83	4.21	35.0	10.4	2.2
3	2280	5.03	3.77	32.0	10.0	2.2
4	2290	5.38	4.44	35.5	12.3	2.1
5	2180	5.58	8.14	34.0	11.6	4.0
6	2370	4.17	4.03	30.0	9.8	2.5
7	1950	4.31	2.76	27.5	8.8	1.5
Mean	2166	5.48	4.46	32.1	10.4	2.2
Group II						
1	2240	9.40	3.95	33.5	11.6	2.8
2	2120	3.59	7.62	26.0	8.8	3.6
3	2300	4.37	3.63	29.0	9.6	4.0
4	2300	4.86	5.40	32.0	10.0	5.3
5	2410	4.81	3.63	28.5	9.2	2.5
6	2390	5.21	5.25	33.0	10.4	1.5
7	1950	5.38	10.17	37.0	11.4	1.6
Mean	2244	5.37	5.66	31.3	10.1	3.0
Group III						
1	2440	4.81	4.54	32.0	10.1	3.0
2	2560	4.90	4.11	33.0	11.0	3.5
3	2360	4.63	8.66	30.5	10.1	1.7
4	2440	5.10	9.36	29.5	9.3	3.6
5	2200	4.95	5.70	31.0	10.9	1.3
6	2650	5.03	6.70	33.5	11.3	2.5
7	2100	4.97	6.70	33.0	10.1	2.6
Mean	2393	4.91	6.53	31.8	10.4	2.6

[1] Red Blood Cells x $10^6/mm^3$.

[2] White Blood Cells x $10^4/mm^3$.

[3] Packed Cell Volume.

[4] Hemoglobin.

[5] Reticulocytes per 1000 erythrocytes.

RAW DATA TABLE IV

FINAL SAMPLES

Animal	RBC Fe mg%	Plasma Fe mg%	RBC Mn ug%	Plasma Mn ug%	MCH[1] uug	MCV[2] u^3	MCHC[3] %
Group I (Control)							
1	29.29	0.42	5.59	4.72	10.76	34.03	31.61
2	30.55	0.25	4.81	5.64	21.53	72.46	29.71
3	29.65	0.25	5.90	6.45	19.88	63.62	31.25
4	26.23	0.16	6.37	3.30	22.86	65.98	34.65
5	27.23	0.85	0.00	6.11	20.77	60.88	34.12
6	27.36	0.18	7.93	3.36	23.47	71.86	32.67
7	19.66	0.83	3.71	5.28	20.39	63.73	32.00
Mean	27.13	0.42	4.90	4.98	19.95	61.79	32.28
Group II							
1	34.51	0.29	7.87	4.09	12.33	35.62	34.63
2	23.58	0.24	10.56	0.00	24.51	72.42	33.85
3	25.55	0.23	3.80	7.93	21.94	66.28	33.10
4	30.42	0.30	4.04	7.35	20.55	65.77	31.25
5	--	--	--	--	19.13	59.25	32.28
6	27.65	0.69	3.14	8.01	19.94	63.28	31.15
7	33.54	0.26	4.04	6.69	21.17	68.71	30.81
Mean	29.20	0.33	5.57	5.67	19.93	61.61	32.43
Group III							
1	29.52	0.15	8.71	0.00	21.00	66.53	31.56
2	29.06	0.18	9.43	4.92	22.45	67.35	33.33
3	20.59	0.65	3.47	5.12	21.79	65.80	33.11
4	17.71	0.57	3.71	6.61	18.23	57.84	31.52
5	25.21	0.22	0.00	5.95	22.02	62.63	35.16
6	25.21	0.39	3.88	9.09	22.44	66.53	33.73
7	22.44	0.19	3.22	6.85	20.32	66.40	30.61
Mean	24.24	0.33	4.63	5.50	21.17	64.72	32.71

[1]Mean Corpuscular Hemoglobin.

[2]Mean Corpuscular Volume.

[3]Mean Corpuscular Hemoglobin Concentration.

RAW DATA TABLE V

GROUP I (CONTROL) FETUS SAMPLES

Fetus	RBC[1]	WBC[2]	PCV[3] %	Hb[4] g%	Reticulocytes[5]	MCH[6] uug	MCV[7] u^3	MCHC[8] %
Doe 1								
No hemogram parameters available.								
Doe 2								
1	2.10	1.70	37.0	9.9	5.4	47.14	176.19	26.76
2	3.73	7.84	44.5	11.8	7.2	31.59	119.14	26.52
3	3.17	1.96	39.0	10.0	5.6	31.50	122.83	25.64
4	3.72	3.27	44.5	11.7	7.3	31.45	119.62	26.29
5	3.54	4.64	43.5	12.0	4.3	33.90	122.88	27.59
6	3.59	3.95	44.0	11.7	7.6	32.54	122.39	26.59
7	3.06	1.99	35.0	9.2	7.8	30.06	114.38	26.28
Doe 3								
1	3.24	2.48	39.5	10.8	--	33.28	121.72	27.34
2	3.61	4.69	44.0	11.8	6.0	32.64	121.71	26.82
3	4.09	4.76	48.0	12.7	5.5	31.05	117.36	26.46
4	3.94	7.96	45.0	11.5	4.5	29.19	114.21	25.55
5	3.10	5.64	36.5	9.2	7.0	29.68	117.74	25.20
6	3.40	5.61	44.0	12.1	10.0	35.59	129.41	27.50
Doe 4								
1	3.45	3.49	44.0	11.4	7.8	32.99	127.35	25.91
2	3.54	4.12	44.5	11.1	8.5	31.35	125.71	24.94
3	3.36	6.69	42.0	11.3	5.4	33.58	124.81	26.90

RAW DATA TABLE V (CONTINUED)

Fetus	RBC[1]	WBC[2]	PCV[3] %	Hb[4] g%	Reticulo-cytes[5]	MCH[6] uug	MCV[7] u3	MCHC[8] %
4	3.38	8.41	46.0	11.9	6.2	35.15	135.89	25.87
5	4.31	7.25	52.0	12.0	5.1	27.84	120.65	23.08
6	3.52	7.23	46.5	13.7	7.8	38.92	132.10	29.46
7	3.22	14.79	39.0	11.0	4.5	34.16	121.12	28.20
8	3.81	14.38	46.0	11.7	7.0	30.71	120.73	25.43
Doe 5								
1	3.22	7.82	47.5	13.1	6.1	40.68	147.51	27.58
2	3.15	9.12	44.0	11.9	8.2	37.78	139.68	27.04
3	3.05	6.27	43.0	11.9	21.0	39.02	140.98	27.67
4	3.23	1.74	43.0	11.5	4.0	35.55	132.92	26.74
5	3.01	1.39	40.5	10.8	6.8	35.82	134.33	26.67
6	3.26	1.59	42.0	11.4	7.0	34.97	128.83	27.14
Doe 6								
1	4.29	2.05	53.0	14.0	6.6	32.60	123.40	26.41
2	4.21	2.42	52.5	14.4	3.1	34.16	124.55	27.43
3	3.91	1.58	50.0	13.5	6.5	34.53	127.88	27.00
4	3.84	1.69	52.0	13.9	4.5	36.20	135.42	26.73
5	3.76	1.90	51.0	13.5	3.5	35.90	135.64	26.47
6	3.91	1.39	47.5	12.9	3.5	32.95	121.33	27.16
7	3.92	2.26	49.0	12.9	5.7	32.91	125.00	26.33
8	3.84	1.69	51.0	13.4	4.4	34.89	132.81	26.27
9	4.15	2.41	51.5	13.2	6.0	31.81	124.10	25.63

RAW DATA TABLE V (CONTINUED)

Fetus	RBC[1]	WBC[2]	PCV[3] %	Hb[4] g%	Reticulo-cytes[5]	MCH[6] uug	MCV[7] u^3	MCHC[8] %
Doe 7								
1	3.79	2.38	47.0	11.9	10.7	31.36	123.85	25.32
2	3.00	1.75	39.5	10.3	9.5	34.28	131.45	26.07
3	3.28	1.36	40.0	10.4	8.9	31.66	121.76	26.00
4	3.16	1.88	38.5	10.0	9.9	31.59	121.64	25.97
5	3.28	1.79	43.0	11.1	8.0	33.84	131.10	25.81
6	4.25	2.75	48.5	11.7	8.1	27.50	113.98	24.12
7	3.92	1.80	48.0	12.1	6.1	30.87	122.45	25.21
Mean								
	3.54	4.22	44.8	11.82	6.9	33.60	127.17	26.39

[1]Red Blood Cells x $10^6/mm^3$.

[2]White Blood Cells x $10^4/mm^3$.

[3]Packed Cell Volume.

[4]Hemoglobin.

[5]Reticulocytes per 1000 erythrocytes.

[6]Mean Corpuscular Hemoglobin.

[7]Mean Corpuscular Volume.

[8]Mean Corpuscular Hemoglobin Concentration.

RAW DATA TABLE VI

GROUP I (CONTROL) FETUS SAMPLES

Fetus	TBW[1] g	Heart[2]	Liver[2]	Kidney[2]	Lung[2]	RBC Fe mg%	Plasma Fe mg%	RBC Mn ug%	Plasma Mn ug%
Doe 1									
1	41.1	0.80	5.62	0.51	0.63	33.33	0.561	15.52	11.57
2	30.2	0.60	6.24	0.56	0.73				
3	35.3	0.63	--	0.58	0.72				
4	35.9	0.82	--	0.60	0.59				
5	37.9	1.01	--	0.54	0.74				
6	41.7	0.62	--	0.50	0.61				
Doe 2									
1	41.7	0.58	5.91	0.57	0.71	32.72	0.748	8.67	6.55
2	37.4	0.63	5.64	0.54	0.84				
3	36.3	0.72	5.37	0.53	0.81				
4	35.7	0.78	5.77	0.64	0.82				
5	36.1	0.76	5.68	0.59	0.87				
6	36.4	0.74	5.63	0.61	0.85				
7	43.9	0.74	6.27	0.65	0.84				
Doe 3									
1	40.0	0.59	5.17	0.57	0.56	39.75	0.598	12.04	7.75
2	46.3	0.71	6.03	0.61	0.72				
3	42.3	0.71	5.21	0.67	0.71				
4	34.8	0.53	5.23	0.53	0.55				
5	36.2	0.68	5.08	0.59	0.60				
6	40.2	0.61	5.52	0.58	0.48				

RAW DATA TABLE VI (CONTINUED)

Fetus	TBW[1] g	Heart[2]	Liver[2]	Kidney[2]	Lung[2]	RBC Fe mg%	Plasma Fe mg%	RBC Mn ug%	Plasma Mn ug%
Doe 4									
1	42.2	0.63	5.51	0.61	0.75	32.72	0.812	10.19	5.55
2	44.7	0.78	5.99	0.55	0.68				
3	33.6	0.67	5.63	0.51	0.67				
4	35.1	0.62	5.05	0.59	0.75				
5	42.5	0.66	5.85	0.51	0.57				
6	44.7	0.56	6.07	0.48	0.69				
7	46.9	0.65	5.70	0.49	0.62				
8	42.3	0.69	5.94	0.55	0.70				
Doe 5									
1	37.3	0.67	6.34	0.48	0.65	31.79	0.898	9.59	6.14
2	30.0	0.75	5.58	0.48	0.72				
3	30.5	0.74	5.78	0.52	0.75				
4	35.1	0.59	5.67	0.47	0.72				
5	37.6	0.60	6.31	0.44	0.68				
6	38.0	0.62	4.63	0.46	0.63				
Doe 6									
1	38.2	0.58	5.68	0.60	0.54	31.46	0.483	13.36	5.43
2	40.7	0.69	5.65	0.68	0.75				
3	35.9	0.57	5.16	0.49	0.74				
4	32.6	0.75	5.17	0.49	0.91				
5	34.9	0.65	5.45	0.50	0.63				
6	34.0	0.48	5.03	0.51	0.65				

RAW DATA TABLE VI (CONTINUED)

Fetus	TBW[1] g	Heart[2]	Liver[2]	Kidney[2]	Lung[2]	RBC Fe mg%	Plasma Fe mg%	RBC Mn ug%	Plasma Mn ug%
7	32.5	0.65	5.67	0.53	0.70				
8	39.5	0.72	6.05	0.49	0.68				
9	38.1	0.80	6.31	0.59	0.53				
Doe 7									
1	25.2	0.55	5.89	0.46	1.22	29.67	0.546	21.15	12.55
2	22.7	0.51	5.50	0.44	1.15				
3	23.0	0.61	5.75	0.56	1.12				
4	23.5	0.79	6.31	0.51	0.94				
5	25.1	0.62	6.03	0.49	0.88				
6	26.3	0.74	6.58	0.47	0.83				
7	24.8	0.60	6.08	0.50	0.97				
Mean									
	36.0	0.66	5.70	0.53	0.73	33.06	0.663	12.93	7.93

[1]Total Body Weight.

[2]Weight as percentage of total body weight.

RAW DATA TABLE VII

GROUP II FETUS SAMPLES

Fetus	RBC[1]	WBC[2]	PCV[3] %	Hb[4] g%	Reticulo-cytes[5]	MCH[6] uug	MCV[7] u^3	MCHC[8] %
Doe 1								
1	6.78	1.29	48.5	13.7	8.9	20.21	71.53	28.25
2	6.22	1.75	47.0	13.9	14.2	22.35	75.56	29.57
Doe 2								
1	3.68	2.84	47.5	13.2	6.7	35.87	129.08	27.79
2	3.95	2.19	49.5	13.9	5.6	35.14	125.16	28.08
Doe 3								
1	3.64	1.76	42.5	12.0	12.0	32.97	116.76	28.23
2	3.07	1.79	47.0	11.5	7.9	37.46	153.09	24.47
3	3.64	1.90	40.0	12.3	9.1	33.79	109.89	30.75
4	3.58	2.51	43.0	12.0	9.5	33.52	120.11	27.91
Doe 4								
1	3.74	2.84	44.0	11.2	8.9	29.91	117.49	25.45
2	3.89	1.85	46.0	11.5	8.8	29.52	118.10	25.00
3	3.60	2.85	42.5	10.5	8.8	29.17	118.05	24.70
4	3.89	4.30	51.5	12.9	8.7	33.16	132.39	25.05
5	4.64	1.75	56.5	13.7	6.7	29.49	121.64	24.25
6	3.95	2.57	46.0	11.6	12.5	29.33	116.31	25.22
7	4.07	2.83	47.0	11.3	9.1	27.76	115.48	24.04

RAW DATA TABLE VII (CONTINUED)

Fetus	RBC[1]	WBC[2]	PCV[3] %	Hb[4] g%	Reticulo-cytes[5]	MCH[6] uug	MCV[7] u^3	MCHC[8] %
Doe 5								
1	2.57	1.44	32.0	8.3	11.4	32.29	124.51	25.94
2	3.85	1.75	43.0	11.5	14.6	29.83	111.54	26.74
3	3.06	1.86	36.0	8.9	26.9	29.08	117.65	24.72
4	3.07	1.19	36.0	9.2	13.8	29.92	117.07	25.55
5	3.57	1.66	42.0	11.2	14.3	31.37	117.65	26.67
Doe 6								
1	3.39	1.66	38.5	10.7	7.9	31.56	113.57	27.79
2	3.02	1.40	34.5	9.5	9.5	31.40	114.05	27.54
3	3.50	2.86	41.0	11.0	11.5	31.38	116.97	26.83
4	3.01	1.81	35.0	9.3	8.7	30.84	116.09	26.57
5	3.25	1.26	38.0	9.9	9.7	30.46	116.92	26.05
6	3.46	1.47	38.0	10.6	11.3	30.63	109.83	27.89
7	3.24	0.63	36.5	10.0	13.2	30.82	112.48	27.40
Doe 7								
1	2.41	2.13	33.0	8.8	8.2	36.44	136.64	26.67
2	3.31	2.15	42.0	10.4	7.2	31.42	126.89	24.76
3	3.50	2.47	39.0	10.6	10.0	30.28	111.43	27.18
4	3.37	2.33	39.0	10.0	8.4	29.67	115.73	25.64

RAW DATA TABLE VII (CONTINUED)

Fetus	RBC[1]	WBC[2]	PCV[3] %	Hb[4] g%	Reticulo-cytes[5]	MCH[6] uug	MCV[7] u^3	MCHC[8] %
5	3.69	2.19	44.0	11.6	10.8	31.44	119.24	26.36
6	3.38	2.26	37.0	10.0	7.2	29.58	109.47	27.03
7	3.83	2.40	41.0	11.0	11.0	28.72	107.05	26.83
Mean	3.67	2.05	41.9	11.1	10.4	30.79	116.33	26.55

[1]Red Blood Cells x $10^6/mm^3$.

[2]White Blood Cells x $10^4/mm^3$.

[3]Packed Cell Volume.

[4]Hemoglobin.

[5]Reticulocytes per 1000 erythrocytes.

[6]Mean Corpuscular Hemoglobin.

[7]Mean Corpuscular Volume.

[8]Mean Corpuscular Hemoglobin Concentration.

RAW DATA TABLE VIII

GROUP II FETUS SAMPLES

Fetus	TBW[1] g	Heart[2]	Liver[2]	Kidney[2]	Lung[2]	RBC Fe mg%	Plasma Fe mg%	RBC Mn ug%	Plasma Mn ug%
Doe 1									
1	50.0	0.64	7.02	0.55	0.60	26.04	0.759	21.25	14.75
2	49.1	0.62	7.35	0.55	0.77				
Doe 2									
1	39.4	0.66	7.26	0.53	0.97	11.53	0.941	26.20	13.82
2	31.6	0.65	6.31	0.57	0.83				
Doe 3									
1	45.8	0.66	8.75	0.52	1.05	34.51	0.784	27.73	10.51
2	38.7	0.61	7.16	0.55	1.04				
3	43.8	0.63	7.18	0.54	0.77				
4	49.3	0.55	7.83	0.51	0.83				
Doe 4									
1	35.2	0.60	6.02	0.52	0.70	35.95	1.599	21.97	10.82
2	37.4	0.60	6.37	0.55	0.82				
3	34.8	0.56	6.33	0.52	0.67				
4	24.7	0.57	4.60	0.48	0.77				
5	25.5	0.60	4.87	0.60	0.83				
6	35.6	0.66	6.22	0.54	0.59				
7	33.8	0.62	6.27	0.54	0.82				

RAW DATA TABLE VIII (CONTINUED)

Fetus	TBW[1] g	Heart[2]	Liver[2]	Kidney[2]	Lung[2]	RBC Fe mg%	Plasma Fe mg%	RBC Mn ug%	Plasma Mn ug%
Doe 5									
1	35.1	0.53	6.64	0.49	0.95	30.60	0.532	15.74	5.99
2	44.0	0.48	7.25	0.57	0.86				
3	38.9	0.50	7.41	0.63	0.95				
4	41.5	0.41	6.82	0.54	0.91				
5	40.5	0.52	7.56	0.65	0.98				
Doe 6									
1	37.3	0.62	6.49	0.55	1.06	25.67	0.426	16.18	6.34
2	44.5	0.63	6.61	0.47	1.37				
3	38.5	0.64	6.90	0.51	0.82				
4	36.6	0.73	7.08	0.43	0.68				
5	33.6	0.73	6.58	0.52	1.21				
6	43.2	0.62	6.52	0.39	1.10				
7	34.7	0.70	6.24	0.55	1.11				
Doe 7									
1	25.2	0.55	5.89	0.46	1.22	30.29	0.393	20.35	7.16
2	22.4	0.52	5.56	0.44	1.16				
3	22.8	0.61	5.81	0.57	1.13				
4	23.2	0.80	6.38	0.51	0.95				

RAW DATA TABLE VIII (CONTINUED)

Fetus	TBW[1] g	Heart[2]	Liver[2]	Kidney[2]	Lung[2]	RBC Fe mg%	Plasma Fe mg%	RBC Mn ug%	Plasma Mn ug%
5	24.9	0.63	6.09	0.49	0.88				
6	26.0	0.75	6.65	0.47	0.84				
7	24.6	0.61	6.14	0.51	0.98				
Mean									
	35.6	0.61	6.59	0.52	0.92	27.79	0.776	21.34	9.91

[1]Total Body Weight.

[2]Weight as percentage of total body weight.

RAW DATA TABLE IX

GROUP III FETUS SAMPLES

Fetus	RBC[1]	WBC[2]	PCV[3] %	Hb[4] g%	Reticulo-cytes[5]	MCH[6] uug	MCV[7] u[3]	MCHC[8] %
Doe 1								
1	3.49	1.22	39.0	10.5	7.6	30.08	111.75	26.92
2	4.27	2.00	48.5	12.8	7.1	29.98	113.58	26.39
3	3.81	1.47	47.5	12.3	8.3	32.28	124.67	25.89
4	3.96	1.34	44.0	11.9	7.3	30.01	110.97	27.04
5	4.06	1.55	46.5	12.1	8.0	29.80	114.53	26.02
6	3.36	1.41	38.5	10.7	9.5	31.80	114.41	27.79
7	3.49	1.96	43.0	11.5	9.1	32.90	123.03	26.74
Doe 2								
1	3.81	1.30	44.0	11.8	8.8	30.93	115.33	26.82
2	3.72	2.07	43.5	12.3	10.2	33.02	116.78	28.27
3	3.85	2.09	44.5	12.1	9.4	31.43	115.58	27.19
4	3.75	1.69	47.0	12.2	7.8	32.49	125.17	25.96
5	3.64	1.46	42.5	11.4	7.6	31.32	116.76	26.82
6	4.02	2.35	48.0	12.5	9.5	31.09	119.40	26.04
7	4.21	2.29	50.5	13.1	8.2	31.12	119.95	25.94
8	4.58	1.46	50.5	13.3	11.2	29.04	110.26	26.34
Doe 3								
1	3.26	1.18	38.0	10.1	10.4	30.98	116.56	26.58
2	3.14	1.15	38.5	10.5	10.4	33.44	122.61	27.27
3	3.35	1.09	42.5	11.0	9.2	32.79	126.68	25.88
4	3.26	0.99	39.5	10.6	9.6	32.51	121.16	26.83

RAW DATA TABLE IX (CONTINUED)

Fetus	RBC[1]	WBC[2]	PCV[3] %	Hb[4] g%	Reticulo-cytes[5]	MCH[6] uug	MCV[7] u^3	MCHC[8] %
5	3.40	1.10	40.0	10.7	9.2	31.47	117.65	26.75
6	3.25	1.17	38.5	10.0	10.2	30.72	118.28	25.97
7	2.95	1.16	36.5	10.1	8.8	34.24	123.73	27.67
8	3.00	1.03	38.0	10.7	20.6	35.67	126.67	28.16
9	3.04	1.57	35.0	10.1	12.0	33.17	114.94	28.86
10	3.05	1.08	36.5	10.0	10.8	32.79	119.67	27.40
Doe 4								
1	3.18	1.00	35.5	10.0	8.1	31.40	111.46	28.17
2	3.15	0.98	37.0	9.6	8.4	30.48	117.46	25.94
3	3.15	1.13	38.0	10.0	10.2	31.69	120.44	26.31
4	3.45	0.87	40.5	10.6	7.0	30.72	117.39	26.17
5	3.14	0.85	38.5	9.7	7.2	30.89	122.61	25.19
6	3.12	0.77	35.5	9.3	10.1	29.76	113.60	26.20
7	2.88	0.63	33.5	9.1	8.0	31.60	116.32	27.16
8	3.08	0.78	38.0	10.1	8.1	32.74	123.18	26.58
Doe 5								
1	4.20	1.20	43.0	12.2	8.1	29.01	102.26	28.37
2	3.86	0.98	39.5	11.3	9.8	29.24	102.20	28.61
3	3.88	1.52	42.0	12.4	16.2	31.96	108.25	29.52
4	3.77	1.07	40.0	11.1	7.9	29.40	105.96	27.75
5	4.25	1.23	45.0	12.7	8.1	29.85	105.76	28.22
6	3.11	0.96	35.0	9.9	8.6	31.83	112.54	28.28
7	4.00	1.17	43.5	11.9	5.9	29.71	108.61	27.36

RAW DATA TABLE IX (CONTINUED)

Fetus	RBC[1]	WBC[2]	PCV[3] %	Hb[4] g%	Reticulo-cytes[5]	MCH[6] uug	MCV[7] u^3	MCHC[8] %
Doe 6								
1	2.63	1.30	31.0	8.8	9.8	33.65	117.87	28.55
2	2.68	0.79	33.0	9.1	9.2	33.95	123.13	27.57
3	2.63	1.53	32.0	8.9	7.8	33.96	121.44	27.97
4	2.25	1.00	29.5	7.9	13.2	35.03	130.82	26.78
5	3.21	1.70	38.0	10.0	19.1	31.15	118.38	26.31
6	3.38	1.82	41.0	11.3	17.4	33.38	121.12	27.56
7	2.76	1.40	32.5	8.8	18.2	32.06	117.75	27.23
8	2.49	1.10	28.0	7.8	15.8	31.32	112.45	27.86
Doe 7								
1	3.52	1.63	39.5	10.9	8.9	30.96	112.21	27.59
2	3.77	2.65	46.5	12.5	9.0	33.16	123.34	26.88
3	2.91	1.79	38.0	10.6	12.2	36.43	130.58	27.89
4	4.32	2.81	51.5	13.6	10.1	31.48	119.21	26.41
5	3.97	3.51	48.0	12.6	6.3	31.70	120.75	26.25

RAW DATA TABLE IX (CONTINUED)

Fetus	RBC[1]	WBC[2]	PCV[3] %	Hb[4] g%	Reticulo-cytes[5]	MCH[6] uug	MCV[7] u^3	MCHC[8] %
6	3.90	2.29	44.5	12.3	9.1	31.50	113.96	27.64
7	3.46	2.08	43.0	12.5	15.1	36.13	124.28	29.07
Mean	3.47	1.44	40.4	10.9	10.1	31.84	117.37	27.14

[1]Red Blood Cells x $10^6/mm^3$.

[2]White Blood Cells x $10^4/mm^3$.

[3]Packed Cell Volume.

[4]Hemoglobin.

[5]Reticulocytes per 1000 erythrocytes.

[6]Mean Corpuscular Hemoglobin.

[7]Mean Corpuscular Volume.

[8]Mean Corpuscular Hemoglobin Concentration.

RAW DATA TABLE X

GROUP III FETUS SAMPLES

Fetus	TBW[1] g	Heart[2]	Liver[2]	Kidney[2]	Lung[2]	RBC Fe mg%	Plasma Fe mg%	RBC Mn ug%	Plasma Mn ug%
Doe 1									
1	47.1	0.59	6.13	0.55	0.89	32.28	0.504	30.41	7.06
2	43.0	0.56	5.77	0.54	0.81				
3	38.1	0.64	6.19	0.58	0.84				
4	42.1	0.59	6.17	0.59	0.64				
5	34.3	0.63	5.71	0.52	0.90				
6	42.3	0.53	5.40	0.48	0.77				
7	41.7	0.60	5.78	0.48	0.75				
Doe 2									
1	31.1	0.52	5.63	0.49	1.05	27.29	0.504	28.07	8.39
2	30.1	0.54	5.34	0.44	0.75				
3	34.5	0.52	6.15	0.50	0.88				
4	31.5	0.53	5.05	0.45	0.95				
5	33.2	0.59	5.98	0.53	1.01				
6	30.1	0.60	5.96	0.41	0.99				
7	24.8	0.63	4.91	0.44	0.84				
8	32.5	0.64	6.44	0.47	0.79				
Doe 3									
1	32.4	0.62	5.69	0.45	1.02	29.43	1.092	25.41	7.37
2	24.2	0.56	4.63	0.60	1.02				
3	22.1	0.68	4.99	0.45	1.05				
4	28.0	0.65	5.63	0.49	1.06				

RAW DATA TABLE X (CONTINUED)

Fetus	TBW[1] g	Heart[2]	Liver[2]	Kidney[2]	Lung[2]	RBC Fe mg%	Plasma Fe mg%	RBC Mn ug%	Plasma Mn ug%
5	29.3	0.68	5.07	0.55	0.74				
6	26.5	0.62	5.26	0.53	1.02				
7	19.7	0.67	4.76	0.57	1.03				
8	30.6	0.62	6.06	0.54	0.79				
9	30.0	0.60	4.77	0.51	0.88				
10	34.2	0.62	6.30	0.52	0.89				
Doe 4									
1	40.3	0.47	6.21	0.34	1.10	25.81	0.349	19.12	6.05
2	38.6	0.47	5.25	0.38	1.05				
3	33.6	0.64	8.15	0.43	1.13				
4	35.3	0.54	5.64	0.40	0.85				
5	35.4	0.58	6.87	0.38	0.92				
6	39.3	0.55	6.40	0.39	0.90				
7	41.4	0.54	6.77	0.37	1.01				
8	40.8	0.61	7.45	0.38	0.62				
Doe 5									
1	33.3	0.45	6.02	0.46	0.79	31.71	0.401	29.16	9.31
2	36.4	0.55	6.92	0.46	0.86				
3	30.8	0.54	5.69	0.50	0.86				
4	34.4	0.59	6.35	0.48	0.84				
5	35.9	0.54	6.90	0.55	0.76				
6	36.9	0.59	6.76	0.51	0.93				
7	32.5	0.65	6.94	0.44	1.00				

RAW DATA TABLE X (CONTINUED)

Fetus	TBW[1] g	Heart[2]	Liver[2]	Kidney[2]	Lung[2]	RBC Fe mg%	Plasma Fe mg%	RBC Mn ug%	Plasma Mn ug%
Doe 6									
1	22.9	0.46	5.59	0.42	1.26	25.83	0.505	26.95	8.85
2	20.3	0.48	6.61	0.46	1.06				
3	19.7	0.46	6.83	0.44	1.10				
4	10.7	0.53	4.52	0.43	1.47				
5	26.4	0.57	8.40	0.48	1.14				
6	28.5	0.53	6.93	0.43	0.96				
7	24.3	0.63	8.19	0.55	1.20				
8	30.9	0.57	8.06	0.43	1.03				
Doe 7									
1	26.6	0.53	6.14	0.47	0.81	28.64	0.557	30.09	0.00
2	23.2	0.47	6.15	0.46	0.87				
3	22.9	0.54	6.30	0.49	0.95				
4	27.1	0.63	6.20	0.51	0.99				
5	25.4	0.55	6.11	0.42	0.95				
6	29.9	0.54	6.13	0.54	0.75				
Mean	31.3	0.57	6.12	0.47	0.93	28.71	0.558	27.03	6.72

[1]Total Body Weight.

[2]Weight as percentage of total body weight.

RAW DATA TABLE XI

DOE LEUKOCYTE DIFFERENTIAL

Animal	Lymphocyte	Neutrophil	Monocyte	Eosinophil	Basophil
Group I (Control) Pre-treatment Samples					
1	193	5	2	0	0
2	131	61	3	1	4
3	180	10	10	0	0
4	166	28	6	0	0
5	172	17	10	0	1
6	173	8	16	1	2
Mean	169	21	8	0	1
Group II Pre-treatment Samples					
1	120	52	17	8	3
2	181	5	10	3	1
3	146	31	14	0	9
Mean	149	29	14	4	4
Group III Pre-treatment Samples					
1	163	19	11	0	7
2	161	27	9	1	2
3	142	42	14	2	0
4	149	38	8	2	3
Mean	154	31	10	1	3
Group I (Control) Final Samples					
1	160	32	4	1	3
2	162	30	6	0	2
3	180	10	10	0	0
4	170	25	5	0	0
5	146	48	6	0	0
Mean	164	29	6	0	1
Group II Final Samples					
1	160	30	7	1	2
2	176	16	4	0	4
3	142	49	4	2	3
Mean	159	32	5	1	3
Group III Final Samples					
1	122	58	14	2	4
2	175	14	9	1	1

RAW DATA TABLE XI (CONTINUED)

Animal	Lymphocyte	Neutrophil	Monocyte	Eosinophil	Basophil
3	126	50	18	2	4
Mean	141	41	14	2	3